BIOLOGICAL SAFETY PLAN

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BIOLOGICAL SAFETY PLAN

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ABBREVIATIONS, ACRONYMS, AND SYMBOLS

Units of measure and some terms commonly understood within the subject disciplines have been abbreviated in the body of this document without callout but are included among the following.

ABSL	animal biosafety level
ALC	authorized laboratory capability
ANSI	American National Standards Institute
APHIS	Animal and Plant Health Inspection Service
BDCF	Baseline Data Collection Facility
BHA	biological hazard assessment
BHM	biohazardous agent or material
BM	biological materials
BMBL	biosafety in microbiological and biomedical laboratories
BSC	biological safety cabinet
BSL	biosafety level
BSL-P	biosafety level – plant
BSO	biological safety officer
BWSA	biohazardous waste storage area
C	degree Celsius
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
DNA	deoxyribonucleic acid
EAB	Environmental Assurance Branch (NASA)
EAR	Export Administration Regulations
EHSO	environmental health and safety officer
EPA	Environmental Protection Agency
ESC	Engineering Services Contract
EtO	ethylene oxide
FAC	Florida Administrative Code
FDA	Food and Drug Administration
GFP	green flourescent protein
GMO	genetically modified organism
HBV	hepatitis B virus
HEPA	high-efficiencey particulate air
HHS	U.S. Department of Health and Human Services
HIV	human immunodeficiency virus
HMI	hazard mitigation inventory
HSO	health and safety officer
IRB	Institutional Review Board
ISC	KSC Institutional Services Contract
ITAR	International Traffic in Arms Regulations
JSC	Lyndon B. Johnson Space Center
KDP	Kennedy Documented Procedure

KEMCON	Kennedy Environmental and Medical Contract
KNPR	Kennedy NASA Procedural Requirement
KSC	John F. Kennedy Space Center
KSC PSCC	KSC Protective Services Cotrol Center
LM	laboratory manager
LMP	lab management plan
Min	minutes
MTC	mutagens, teratogens, carcinogens, and reproductive toxins
NaClO	sodium hypochlorite (bleach)
NASA	Nation Aeronautics and Space Administration
NE	KSC NASA Engineering Directorate
NIH	National Institutes of Health
NLR	no license required
NPR	NASA Procedural Requirement
NSF	National Sanitation Foundation
OHF	Occupational Health Facility
OHS	Occupational Health and Safety
OSHA	Occupational Safety and Health Administration
PFA	pararomaldehyde
PI	principal investigator
PIR	pollution incident reporting
PLN	plan
PPE	personal protective equipment
PSCC	KSC Protective Services Control Center
Psi	pounds per square inch
PWQ	Process Waste Questionnaire
PWQTRP	Process Waste Questionnaire technical response package
rDNA	recombinant deoxyribonucleic acid
RG	risk group
RNA	ribonucleic acid
SHRB	Safety and Health Review Board
SOP	standard operating procedure
UB	KSC NASA Exploration Research and Technology Programs Directorate
UG	user guide
USA	Unided States of America
USDA	United States Department of Agriculture
w/v	weight/volume
WG	working group
WMA	waste management authority
XNA	xenonucleic acid

1. INTRODUCTION

1.1 Purpose

Biosafety applies knowledge, techniques, and equipment to prevent personal, laboratory, and environmental exposure to potentially infectious agents or other biohazards. This Biosafety Plan has been developed under the supervision of the NASA NE/UB Safety and Health Review Board (SHRB) to provide guidance for activities involving biological materials (BMs) in NE- and UB-managed areas at Kennedy Space Center (KSC). The goals of establishing this Biosafety Plan is to provide guidance for protecting personnel from exposure to infectious agents; preventing environmental contamination; providing an environment for research while maintaining a safe work place; and complying with applicable NASA, Federal, State, and local requirements.

1.2 Scope

The policies and procedures defined here apply to the personnel involved in the research activities associated with BMs, including infectious agents and toxins, as well as recombinant deoxyribonucleic acid (rDNA) molecules, in NE/UB laboratories under the oversight of SHRB which are not covered by NASA programs. These laboratories are listed in the file "Designated SHRB KSC_Labs_with_Facilities" on the NE SharePoint site. This plan also supplements KSC-UG-1904, NASA Employee Exposure Control Plan for Bloodborne Pathogens, to protect employees who handle bloodborne pathogens for experimental research. Affected personnel include NASA and contractor employees, visiting researchers, interns, and nonlaboratory workers who support or conduct work in these listed laboratories

Only Biosafety Level (BSL)-2/2P and Animal Biosafety Level (ABSL)-2 and below (including corresponding recombinant rDNA molecules) BMs shall be allowed in KSC laboratories or facilities. Using select agents and toxins listed in the Code of Federal Regulations: Select Agent Standard (reference http://www.selectagents.gov/SelectAgentsandToxinsList.html) is prohibited at KSC.

2. APPLICABLE DOCUMENTS

The following documents form a part of this document to the extent specified herein.

The latest versions of KSC documents (KDPs, KNPRs, KSC-PLNs, and KSC-UG-1904) can be found at: https://tdsearch.ksc.nasa.gov/.

The latest versions of ESC documents can be found at: https://adfs2.ksc-esc.com/.

The latest versions on NPRs can be found at: https://nodis3.gsfc.nasa.gov/main_lib.html.

Center for Disease Control	Federal Select Agent Program, Select Agents and Toxins (http://www.selectagents.gov/SelectAgentsandToxins.html)
Florida Administrative Code (FAC) 64E-16	Biomedical Waste (http://www.floridahealth.gov/environmental-health/biomedical-waste/_documents/64E16_1.pdf)
HHS Publication No. (CDC) 21-1112	Biosafety in Microbiological and Biomedical Laboratories (BMBL) (http://www.cdc.gov/biosafety/publications/bmbl5/index.htm)
KDP-KSC-F-2616	KSC Councils, Boards, And Committees Charter Form
KDP-KSC-P-1473	Safety and Mission Assurance Mishap Notification Process
KDP-KSC-P-3001	Warning, Alerting, and Evacuation
KDP-KSC-P-5458	Capabilities Determination Process
KNPR 1820.4	KSC Respiratory Protection Program
KNPR 1840.19	KSC Industrial Hygiene Program
KNPR 8500.1	KSC Environmental Requirements
KNPR 8715.5	KSC Personal Protective Equipment (PPE) Program Procedural Requirements
KSC-ESC-3000	ESC Safety, Health, and Environmental Manual
KSC-PLN-1800	Engineering Directorate (NE) and Engineering Services Contract (ESC) Laboratory Safety and Chemical Hygiene Plan
KSC-PLN-2322	NE/UB Laboratory, Shop, and Test Facility Management Plan
KSC-UG-1904	NASA Employee Exposure Control Plan for Bloodborne Pathogens

Morbidity and Mortality Weekly, 2011	Guidelines for Biosafety Laboratory Competency, CDC and the Association of Public Health Laboratories (https://www.cdc.gov/mmwr/pdf/other/su6002.pdf)
NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules	(http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html)
NPR 1800.1	NASA Occupational Health Program
NPR 8621.1	NASA Procedural Requirements for Mishap and Close Call Reporting, Investigating, and Recordkeeping
NSF/ANSI 49-2008, Annex F	Biosafety Cabinetry: Design, Construction, Performance, and Field Certification (http://standards.nsf.org/apps/group_public/download.php/3604/NSF_49-08e-rep-watermarked.pdf)
OSHA Standard 1910.1047	OSHA Regulations Code of Federal Regulations, Title 29, Ethylene oxide-1910-1047 (https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=standards&p_id=10070)
OSHA Standard 1910.1048	OSHA Regulations Code of Federal Regulations, Title 29, Formaldehyde-1910-1048 (https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_id=10075&p_table=STANDARDS)
The Belmont Report, Office of the Secretary, The National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research	Ethical Principles and Guidelines for the Protection of Human Subjects of Research (http://www.hhs.gov/ohrp/regulations-and-policy/belmont-report/index.html)

3. DEFINITIONS

Animal Biosafety Level (ABSL): a level from 1 to 4 designated in ascending order by degree of increasing level of protection and containment required to prevent personnel, the environment, and the community from exposure to hazardous biological materials. Animal Biosafety Level 1 (ABSL-1) through Animal Biosafety Level 4 (ABSL-4) address combinations of practices, safety equipment, and facilities for experiments with animals infected with agents that cause, or may cause, human infection. Only ABSL-1 and ABSL-2 agents shall be used in KSC facilities.

Authorized Laboratory Capability (ALC) and Hazard Mitigation Inventory (HMI): The ALC, along with the HMI, provides the information on how to safely perform work in the designated areas. All work will be performed within the context of an ALC.

Biohazardous Agent or Material (BHMs): A biohazardous agent is biological in nature, capable of self-replication, and is capable or potentially capable of producing deleterious effects upon biological organisms. A biohazardous material is any material that contains or has been contaminated by a biohazardous agent.

Biological Materials (BMs): There are three categories of biological materials: 1. living organisms, including BHMs; 2. biological toxins; and 3. nonliving materials which enable reproduce themselves once being introduced into a host cell, (such as viruses, vectors, cell organelles. Representative examples of BMs include bacteria, fungi, algae, protozoa, eukaryotic cells, animal cell lines, hybridomas, plants, plant cells, seeds, lichens.

Biomolecules: The molecules that are present in living organisms, including large macromolecules such as proteins, carbohydrates, lipids, and nucleic acids, as well as small molecules such as primary metabolites, secondary metabolites, and natural products. Biomolecules are usually endogenous but may also be semisynthetic or synthetic.

Biological Toxins: Toxic biomolecules produced by bacteria, fungi, protozoa, insects, animals, or plants.

Biosafety Level (BSL): A level from 1 to 4 designated in ascending order by degree of increasing level of risk and associated protection and containment required to prevent personnel, the environment, and the community from exposure to hazardous biological materials.

Biosafety Level – Plant (BSL-P): A level from 1 to 4 designated in ascending order specific for whole plants by degree of increasing level of protection and containment required to prevent personnel, the environment, and the community from exposure to hazardous biological materials.

Biological Safety Cabinet (BSC): Air-flow-controlled cabinet that uses a high-efficiency particulate air (HEPA) filter to protect laboratory personnel and the environment, as well as the BMs.

Bloodborne Pathogen: A pathogenic microorganism that is present in human blood and can cause disease in humans. Examples of these pathogens include hepatitis B virus (HBV) and the human immunodeficiency virus (HIV).

Containment: A method for managing BMs in a laboratory to reduce or eliminate exposure of personnel and the outside environment.

Genetically Modified Organism (GMO): An organism and its offspring containing artificially altered genetic material that cannot be found in nature.

KSC Protective Services Control Center (KSC PSCC): The emergency dispatch service for KSC. The PSCC receives all emergency calls at 911 (land lines) and 321 867-7911 (cell phones).

Institutional Review Board (IRB): The KSC Human Research IRB Working Group (WG) (authorized by KDP-KSC-F-2616) that approves, monitors, and reviews biomedical and behavioral research involving human subjects. The purpose of the IRB is to ensure appropriate steps are taken to protect the rights and welfare of humans participating as subjects in a research study.

Laboratory Manager (LM): The work area manager responsible for one or more assigned work area(s). See the KSC-PLN-2322, NE/UB Laboratory, Shop, and Test Management Plan, Section 3.1.12.

Mutagens, Teratogens, Carcinogens and Reproductive Toxins (MTC): Hazardous materials that can cause serious health problems (e.g., cancer, birth defects, sterility, and genetic mutations) in workers or their children.

Personal Protective Equipment (PPE): The specialized clothing or equipment worn for protection against a hazard.

Principal Investigator (PI): The primary researcher with overall responsibility for a research project or program.

Process Waste Questionnaire (PWQ): The document that is submitted by a generator of biohazardous or chemical waste to identify each waste process, including waste characteristics, volume, contacts, etc.

Process Waste Questionnaire Technical Response Package (PWQTRP): The waste guidance document provided by the Waste Management Authority (WMA) that identifies the results of the hazard determination conducted in response to a PWQ. The PWQTRP provides technical information necessary to containerize, label, and manifest a hazardous, universal, or controlled waste.

Recombinant DNA (rDNA): The DNA molecules formed by laboratory methods of genetic recombination (such as molecular cloning) to create sequences that would not otherwise be found in the genome of the original organism.

Selected Biological Agents and Toxins: These biological agents and toxins have been determined by the Animal and Plant Health Inspection Service (APHIS) and the Centers for Disease Control and Prevention (CDC) to have the potential to pose a severe threat to both human and animal health, to plant health, or to animal and plant products (http://www.selectagents.gov/SelectAgentsandToxinsList.html).

Sharps: An object capable of puncturing, lacerating, or otherwise penetrating human skin.

4. MANAGEMENT AND RESPONSIBILITY

Maintaining biosafety is a cooperative effort, involving the SHRB, NASA biological safety officer (BSO), NASA/ESC hygiene and safety officers (HSOs), lab managers (LMs), principal investigators (PIs), laboratory personnel, and visiting researchers. Each individual involved in using BMs or conducting tasks associated with BMs is responsible for complying with this Biosafety Plan. The contact information of BSO, HSOs, and Occupational Health Facility (OHF) for consulting on BM-related questions and concerns is listed in the SHRB Contacts List located on the NE SharePoint site. In addition to the responsibilities of individuals and organizations described in KSC-PLN-2322, specific responsibilities related to biological safety are listed in the following paragraphs.

4.1 Safety and Health Review Board (SHRB)

The SHRB will review and approve KSC-PLN-1801, Biological Safety Plan.

4.2 Biological Safety Officer (BSO)

The BSO shall have training in the life sciences and understand laboratory and clinical practices, the regulatory and consensus body knowledge on how to properly manage the material(s), the physiological action of BMs, and the means to recognize, evaluate, and control those hazards. The BSO

- ensures that biological safety documentation for the work areas meets the requirements of NPR 1800.1 and KNPR 1840.19,
- participates in the SHRB process as a subject expert and ensures that this document and associated BM-related operation procedures are maintained,
- reviews and approves the use of BMs,
- reviews and approves the NASA procurement of BMs (for ESC, this function is performed by the EHSO),
- ensures the list of BMs in use or in storage is maintained, and
- provides consultation to the work area manager for the safe use of BMs and biological safety concerns.

4.3 Laboratory Managers (LMs)

The LMs will coordinate the work of PIs and laboratory personnel to maintain biosafety in their laboratory. In addition to the responsibilities for LMs described in KSC-PLN-2322, they shall:

- ensure work involving BMs is conducted in accordance with established policies, general guidelines, and standard procedures described in this document,
- review and approve BM-specific procedures in coordination with PI and BSO, and
- ensure prompt reporting of any BM-related injuries, potential exposures, or illnesses to the supervisor and BSO.

4.4 Laboratory Personnel

Personnel performing work with BMs are responsible for operating in a safe manner. Ultimately, each individual is responsible for their own safety. Contact the BSO for any concerns or questions regarding the safety of BMs. Lab personnel shall:

- perform work in accordance with established policies and guidelines described in this document and specific laboratory ALCs, HMIs, and SOPs,
- wear and maintain PPE required for performing each task and understand the limitations of PPE,
- use the engineering controls required for performing each task, and
- complete required training listed in the ALCs.

4.5 Occupational Health Facility (OHF)

The OHF will:

- provide medical consultation or examination following workplace injuries, illnesses, and incidents,
- evaluate effected worker's current medical conditions and determine medical services required to permit safe performance of work duties, and
- assist in developing surveillance programs for work involving specific BMs.

5. BIOLOGICAL HAZARD ASSESSMENT

The biological hazard assessment (BHA) process has been integrated into the existing HMI hazard assessment process. The required information for a BHA can be found in KSC Form 50-211, Laboratory Hazard and Mitigation Inventory, and is listed in the Appendix A. Before conducting work with BMs, the PI and LMs shall have approved an ALC/HMI that addresses all the safety mitigations, including biosafety requirements. Requests for new BM-related activities

will be reviewed by the PI, the LM, the BSO, and the HSOs to determine if there are any additional hazards or changes to the hazard levels compared to previously authorized work. The review will comply with KSC-PLN-2322 and KDP-KSC-P-5458. Written procedures on handling specific BMs shall incorporate the required hazard mitigations described in the HMI. .

6. TRAINING

Training is required to comply with Occupational Safety and Health Administration (OSHA) regulations and KNPR requirements. Training requirements are determined by both the LM and PI, in consultation with the HSOs and BSO, and shall be documented in the LMP or ALCs. Training on handling BMs should include, at a minimum:

- Basics of Biosafety (SATERN course; JSC-OHS-BOB)
- Familiarization with the this Plan (KSC-PLN-1801)
- Biohazardous Waste Management.
- Bloodborne Pathogen (BBP) training is required for work with blood or other potentially infectious materials as defined in KSC-UG-1904, NASA Employee Exposure Control Plan for Bloodborne Pathogens.
- ESC employees supporting research activities with blood or other potentially infectious materials shall comply with KSC-ESC-3000, Chapter 6, Bloodborne Pathogens.

For projects involving human subjects, additional training may be required and shall be coordinated with the IRB and BSO.

7. INSPECTION OF LABORATORY FACILITIES

The inspection schedule for the areas handling or storing BMs shall be compliant with the inspection schedule described in this document. The KSC-specific Inspection Checklist in Appendix B is modified from the BSL-2 and NIH BL-2 checklist provided by the CDC and USDA (https://www.selectagents.gov/checklists.html).

8. EMERGENCY AND SPILL CONTROL

Laboratory personnel handling hazardous materials should be familiar with and comply with Center and laboratory emergency procedures documented in the KDP-KSC-P-3001 and KNPR 8500.1. This section only specifies the emergency and spill control procedures for incidents involving BMs.

8.1 Biological Spills

The severity of the hazard associated with a biological spill is a function of the volume of the spill, the pathogenicity of the agent, and its concentration within the spilled material.

When a spill occurs, the appropriate response should consider the protection of employees; preventing release of viable biological agents outside of the area handling BMs; and cleanup/decontamination of the area.

To control a biological spill, follow the cleaning procedures listed in Appendix C using universal precautions and good laboratory practice. If the spill cannot be safely controlled, evacuate the affected area (decontaminate personnel as soon as possible); protect the affected area; and contact BSO and HSO for guidance of appropriate spill response. For a nonemergency spill, call the ISC Duty Office (861-5050) to clean it up. For an emergency spill, call 911 from a land line or 321-867-7911 from a cell phone. When reporting an emergency spill, please include the following information:

- Statement that this is an emergency spill.
- Your name and phone number.
- Extent of injuries, fire, or explosion.
- Specific location of spill, facility name, and number.
- Room number (if applicable).
- Type of material released, whether it is genetically modified.
- Estimated quantity released, in volume (gallons) or surface area covered.
- Rate of release.
- Whether or not the spill is contained.
- Worst-case credible quantity of material that could be released.
- Potential risk to human health or the environment, if known.

8.2 Basic Biological Spill Kit

Laboratories working with BMs shall have a basic biological spill kit ready to use prior to starting operations. The basic kit can be purchased from a vendor or can be assembled with materials already used in the laboratory. The spill kit shall contain adequate materials to clean up the largest credible spill for the work performed. The laboratory ALCs or HMIs shall describe the availability and accessibility of an appropriate biological spill kit and its contents.

A basic spill kit at a minimum contains the following:

- Disinfectant (e.g., bleach 1:10 dilution from 6% stock [NaClO] solution, prepared before each use)
- Absorbent material (e.g., paper towels)
- Waste container (e.g., biohazard bags, sharps containers)
- Personal protective equipment (e.g., lab coat, gloves, eye and face protection)
- Mechanical tools (e.g., forceps or tongs)

8.3 Potential Exposure Response Protocols

In the event of a potential exposure to a BM, the affected person should immediately report to the OHF to seek medical attention. When the affected person is seen by a medical professional, the affected person should tell them that this incident occurred in a laboratory setting and that the occupational health physician should be notified. The affected person should provide the medical professional with as much detail as possible on the BM to which they are exposed and should state if it is genetically modified.

If potential exposure is associated with the eyes, mucosa, or an open wound, immediately decontaminate the body part, and report as soon as possible to the OHF to seek medical attention. After business hours, call 911(from a landline) or 321-867-7911 (from a cell phone).

8.4 Reporting

Employees are required to report any close calls, mishaps, injuries, illnesses, or potential biohazard exposures to their immediate supervisor in accordance with KDP-KSC-P-1473. Accident records will be completed by the supervisor in accordance with NPR 8621.1. In the event of a major biological spill, injury, or potential BM exposure, LMs, BSO, HSOs, and supervisors shall be notified. The BSO shall follow up if any additional information is needed for reporting and investigating, determining what actions will be taken to prevent a future spill in the affected laboratory, and may require completion of a report for NIH. A complete record shall be kept by the LMs, the BSO; and the OHF, if potential exposure or injury occurs.

If the spill is released to the environment, notify the NASA Environmental Assurance Branch (NASA EAB) immediately so that they can notify the State Emergency Response Commission.

The supervisor shall work with the employees to fill out a KSC-21-555 form, KSC Pollution Incident Reporting and Notification (PIR), and submit it to NASA EAB. The initial report must be submitted within three working days of the incident.

9. BIOSAFETY GENERAL PRINCIPLES

Biosafety can be accomplished by using appropriate containments that allow BMs to be safely manipulated to reduce the potential exposure. The primary containment protects personnel and the immediate laboratory environment through good microbiological technique/laboratory practice and the use of appropriate safety equipment. The secondary containment protects the external environment from exposure to BMs through a combination of facility design and operational practices.

9.1 Biosafety Levels

Different levels of physical containment can be achieved by combining laboratory practices, containment equipment, and special laboratory design. Currently, there are four Biosafety Levels (BSL 1-4) used to define the level of containment necessary for corresponding levels of BMs to protect personnel and the environment. This plan focuses on the requirements for handling the BSL2 and lower BMs that are allowed at KSC. A summary of the BSL 1-2 classification, containment, and facility requirements for safely handling corresponding Risk Group Level 1-2 (RG 1-2) organisms, toxins, or rDNA can be found in the Appendix D.

9.2 Plant Biosafety

The risk assessment for the plant BSL is based on specific sessions of the NIH Guidelines regarding plant research. The plant biosafety categories are summarized and listed in Appendix E. The general facility biosafety management guideline for plant study is summarized in Appendix E. The containment requirements and exposure control measures are consistent, whenever applicable, to those applied for BSL-1/BSL-2 agents.

The PIs will obtain permits from regulatory authorities, i.e., the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS), for research projects involving importing and transporting designated plants, plant products, and soil into the U.S.A., through the U.S.A., importing plant pests and biological control organisms into the U.S.A., and moving plant pests and biological control organisms between states. The Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) may also be involved in the regulation of transgenic plants. The PI must comply with any additional regulations.

9.3 Using Biomolecules

9.3.1 Recombinant DNA/RNA Biosafety

Work with genetically modified organisms shall be done in compliance with the National Institute of Health Guidelines for Research Involving Recombinant DNA Molecules. These guidelines classify recombinant DNA experiments into four levels of containment (identical to those for infectious microorganisms) based on the hazard of the organism and the procedures and quantities being used. The classification of BSL-1 and BSL-2 research related to rDNA and associated GMOs is listed in Appendix C. The containment requirements and exposure-control measures are identical to those applied for BSL-1/BSL-2 agents.

9.3.2 Working with Viral Vectors

Viruses and viral vectors are widely used in molecular biology. It is important for lab personnel to understand the origins of these tools and the potential implications of their use; including clinical features, epidemiology, treatment, laboratory hazards, personal protective equipment (PPE), and disinfection. Suggested biosafety containment levels are exactly the same as those used for the corresponding virus. Use of a higher-level containment facility may be required in some cases, depending on the specific properties of the vector or insert. Special care should be given to designing and handling virus vectors containing genes that make growth-regulating products, products released into the circulation, and products that may have a general effect on the host's immune system. Work with any viral vector requires approval from the BSO and the SHRB.

9.3.3 Biological Toxins

Laboratory work with most toxins, in amounts routinely applied to the experiments, can be performed safely with minimal risk to the worker and negligible risk to the surrounding community. Toxins do not replicate, are not infectious, and are difficult to transmit mechanically or manually from person to person. The main risks are accidental exposure by direct contamination of mucous membranes; by inadvertent aerosol generation; and by needle-sticks or other accidents that may compromise the normal barrier of the skin. Biological toxins can be handled using established general guidelines for BMs described in this plan.

9.4 Handling Unfixed Animal Tissues

Hazards associated with handling unfixed animal tissues fall into the following three categories:

- Physical injuries, such as scratches or other direct injuries.
- Allergic hazards associated with breathing or contacting allergens found in animal tissues.
- Zoonotic diseases potentially transmitted between animal tissues and humans.

The containment requirements and exposure-control measures are treated as to those applied for BSL-1/BSL-2 agents.

9.5 Mutagens, Teratogens, Carcinogens, and Reproductive Toxins

The use of mutagens, teratogens, carcinogens, and reproductive toxins shall comply with KSC-PLN-1800. Exposure shall be minimized by using engineering controls such as fume hoods or BSCs, using PPE properly, using only the minimum amount necessary, and clearly labeling containers with the hazards present to warn other users to take precautions.

10. BIOLOGICAL HAZARD CONTROL MEASURES

Personnel working with BMs in KSC laboratories and facilities that are not covered by NASA programs shall adhere to this Biosafety Plan, the NIH guidelines, and the CDC's guidelines. The most important element in maintaining a safe work environment is strict adherence to good microbiological and laboratory practices and techniques. All employees working with BMs must be aware of the potential risks. Employees must also be trained and proficient in the practices and techniques required for independently handling such material.

Because the potential for biosafety impact is unknown for most BMs, universal precautions recommended by the CDC and NIH shall be followed. The minimum precautions for procedures shall be followed regardless of any lack of evidence of BMs. Primary hazards to personnel working with BSL-2 BMs relate to accidental percutaneous, mucous membrane exposures, or ingestion of BMs.

The general guidance listed below must be followed in laboratories handling BMs:

- Processing or analysis related to BMs shall be conducted in laboratories designed and furnished with equipment appropriate for the BSL level.
- Standard microbiological work practices shall be used. Most procedures will require the use of engineering controls like biological safety cabinets (BSC) for BSL-2/-2P BMs.
- Personnel shall wear PPE appropriate for the potential exposure. Minimum PPE required for working with BSL-2/2-P BMs include a lab coat, eye protection, and gloves.
- Use of needles and other sharps shall be avoided whenever possible.
- Employees who have open lesions, dermatitis, or other breaks in the skin compromising skin barrier protection need special attention when performing work with BMs. Appropriate gloves, such as medical exam gloves, must be worn. These employees may need to use additional barrier protection until the condition resolves.
- Laboratory doors must be kept closed for processing BSL-2/2-P BMs. Access to certain work areas may be restricted during the use of hazardous materials or special procedures.

10.1 First Aid Following Potential Exposure

A potential exposure is defined as a scratch, laceration, or puncture wound caused by potentially contaminated equipment, mucous membrane exposure to potentially contaminated tissues, cell cultures, and body fluids. Following a potential exposure, the first most important step is to immediately wash the site with soap and water for 15 minutes.

If an eye exposure occurs, flush the eye for 15 minutes. Report immediately to the OHF and contact the LMs, the BSO, and the supervisors as instructed in paragraph 8.4.

10.2 Engineering Controls

Engineering controls are various types of equipment used to minimize contact with a hazard. Common engineering controls for biological materials include biosafety cabinets, sharps containers, centrifuge safety cups, and vacuum-line high-efficiency particulate air (HEPA) filters. Engineering controls are the preferred control measure. The LMs shall identify appropriate engineering controls in the ALC/HMIs and generate specific work procedures governing their usage for specific agents, if necessary.

10.2.1 Biological Safety Cabinets (BSCs)

BSCs are local exhaust or filtration systems used to generate a ventilated, enclosed work space intended to capture, contain, and exhaust or filter harmful BMs. BSCs are designed to provide personnel, environmental, or product protection when appropriate practices and procedures are followed. Two kinds of BSCs, Class I and Class II, have been developed to meet BSL-1 and BSL-2 biological research needs.

BSCs should not be confused with other laminar flow devices or clean benches; in particular, horizontal-flow cabinets, which direct air towards the operator. These benches protect the product but do not protect the operator. Laboratory personnel should be trained in the correct use and maintenance of BSCs to ensure that personnel and product protection (where applicable) is maintained. The correct location, installation, and certification of the BSC is critical to containing infectious aerosols. A brief description of different types of BSCs and maintenance procedures is included as Appendix F. When properly used with good laboratory practices and procedures, BSCs are effective in containing and controlling particulates and aerosols.

All BSCs shall be inspected at least annually and certified by trained and accredited service personnel according to the National Sanitation Foundation (NSF) Standard 49, Annex F. Inspection and recertification is required if the cabinet is relocated or after major repairs, filter changes, etc. For general guidance on the safe and effective use of BSCs refer to the CDC/NIH guidelines

10.2.2 Ducted Exhaust Air Ventilation System

The directional airflow created by the ducted exhaust air ventilation system shall draw air into the work area and discharge the exhaust to the outside, away from occupied areas and air intakes. The proper direction of the airflow shall be checked by laboratory personnel for negative airflow, if applicable. Contact the HSOs and the BSO if the airflow is determined to be positive.

10.2.3 Loop Sterilizers

Using a shielded electric incinerator or hot-bead sterilizer minimizes aerosol production during loop sterilization. Disposable plastic loops and needles are highly recommended for working with BMs.

10.2.4 Eyewash Stations

Eyewash stations are required in laboratories where BMs are stored and where employees perform tasks involved BMs. Eyewash stations shall be inspected weekly.

10.3 Medical Consultation

Employees may consult with OHF physicians to discuss information about health hazards associated with BMs or chemicals that affect the health of reproductive organs, fertility, or embryonic and fetal development. Consultation can include the Center policy, occupational and medical histories, discussion of workplace hazards of concern, and work practices that may be followed that can reduce the hazard exposure. Medical examinations may be required for a specific exposure or job assignment (e.g., bloodborne pathogens, animal handlers, etc.).

10.3.1 Special Considerations for Reproductive and Developmental Hazards

Employees who declare an actual, suspected, or intended pregnancy, or others with concerns regarding potential exposure to reproductive or developmental hazards in the workplace, shall complete KSC Form 28-1908V2, the Reproductive and Developmental Health Hazard Questionnaire. The employee shall provide the Reproductive and Developmental Health Hazard Questionnaire to their personal physician for review. The employee is responsible for providing their physician's written limitations to their supervisor. The work restrictions or limitations shall be implemented in accordance with the employer's safety and personnel policies. Specific requirements regarding management of reproductive and developmental hazards may be found in KNPR 1840.19, KSC Industrial Hygiene Programs.

10.3.2 Immunodeficient or Immunosuppressed Employees

Any employee who handles biological agents and is immunodeficient or immunosuppressed should consult with their personal physician to determine any special conditions or limitations for work with BMs. It is the responsibility of the employee to provide the physician's written

limitations to their supervisor. The work restrictions or limitations shall be implemented in accordance with the employer's safety and personnel policies.

10.4 Labeling

A biohazard label is required for areas or equipment where biohazardous agents or materials (BHMs) are handled or stored. Post the label at the main entrance door to laboratories, and on equipment like refrigerators, incubators, and transport containers (see Figure 1).

10.4.1 General Labeling and Signage Requirements

Place biohazard signs and labels (see Figure 1) in laboratory or storage areas as follows.

- Use laboratory safety plaques (front door signs) to ensure laboratories where BMs are stored or used are clearly labeled.
- Clearly label biosafety cabinets where biohazards are used.
- Label refrigerators, freezers, centrifuges, and incubators where biohazards are used.
- Evaluate other pieces of equipment and assess them for risk. Label items with the biohazard symbol if they are at risk of being contaminated during laboratory activities.
- Clean and decontaminate equipment items with biohazard labels prior to removal from the laboratory for service or disposal. Information on the decontamination procedure must accompany the equipment

10.4.2 Sample Container Labeling and Storage Requirements

Clearly label containers or racks to identify the contents. Secondary containment shall be applied to BSL-2 BMs. If there is a large quantity of smaller containers of the same agent, labeling the storage container, tray, or cupboard will suffice.

As shown in Figure 1, a variety of different biohazard labels are available through the laboratory supplies vendor.

Figure 1. Biohazard Sign Examples

10.5 Work Practice

10.5.1 General Laboratory Work Practices

- Eating, drinking, smoking, handling contact lenses, or applying cosmetics is permitted only in designated areas.
- Minimize the chances of creating a splash or aerosols when performing procedures.
- Wearing safety glasses with side shields or goggles covered by a face shield is required in situations where splashes or aerosols of biological materials may occur.
- Clean and decontaminate work surfaces following the spill-control procedure described in Appendix C and the decontamination procedure described in paragraph 11 after any spill or release of viable material and at the end of each work day.
- After removing gloves and before leaving the laboratory, personnel must wash their hands after handling viable BMs.

10.5.2 Pipettes and Pipetting Aids

Use the following precautions when pipetting.

- Confine pipetting of fluids containing BMs in a biosafety cabinet if possible. If pipetting is done on the open bench, use absorbent pads on the bench and place disinfection reagent near to reach.
- Mouth pipetting is strictly prohibited. Use mechanical pipetting.
- Always use cotton-plugged disposable pipettes when pipetting fluids containing BMs.
- Carefully prepare BM mixtures by suction and expulsion through a pipette. Use “to deliver” pipettes rather than those requiring “blowout.” Avoid discharging BMs from a pipette at a height to prevent creating aerosols.
- Discard contaminated pipettes in appropriately sized biohazard sharp containers.
- When work is performed inside a biosafety cabinet, place pans or sharps containers for contaminated glassware inside the cabinet while in use.

10.5.3 Needles, Syringes, and Other Sharps

Handle syringes and hypodermic needles with extreme caution to avoid accidental injection and aerosol generation. Generally, the use of syringes and needles should be restricted to procedures for which there is no alternative. The use of safe needles (needle-locking syringes and disposable syringe-needle units) is highly recommended.

- Use syringes that are designed with a safety shielding needle, needleless systems, and other safe devices when appropriate.

- Dispose of ALL needles, syringes, and other sharps (used or unused) in appropriate sharps containers. Do not discard syringes, needles, and other sharps into laboratory waste receptacles or pans containing pipettes or glassware.
- Do not overfill sharps containers (2/3 filled should be considered full).
- DO NOT RECAP NEEDLES. If a needle MUST be recapped, use a mechanical device or the one-handed scoop method.
- DO NOT BEND, CUT, REMOVE OR BREAK NEEDLES and other sharps.
- Use "safe" needles/syringes, i.e., needle-locking syringes and disposable syringe-needle units, or sharps when applicable.
- Plan work to avoid quick and unnecessary movements while working with syringes.
- Where appropriate, fill or immerse syringes in disinfectant prior to discarding them into a sharps container.
- Use separate containers for disposable and nondisposable syringes and needles.
- Examine glass syringes for chips and cracks and needles for barbs and plugs prior to sterilization and before use. **Use glass syringes as a last resort. Disposable syringes and needles or safe needle/syringe technology is preferred.**
- Fill syringes carefully to minimize air bubbles and frothing of the inoculum.
- Work in a biosafety cabinet whenever possible.
- Expel excess air, liquid, and bubbles from the syringe vertically into a cotton pledget moistened with proper disinfectant, or into a container with appropriate media, reagents, or disinfectant.
- Avoid contaminating the hub of the needle when filling from a test tube, , this may result in transfer of infectious material to the hands.

10.5.4 Frozen Sections of Unfixed BMs

Because freezing tissue usually does not inactivate infectious agents, frozen sections of unfixed human tissue and animal tissue infected with an etiologic agent or with an unknown biohazard may pose a risk. Wear gloves when preparing frozen sections or performing analyses. Solutions used to stain potentially infected frozen sections, containers, and forceps are considered contaminated when working with frozen sections of unfixed BMs.

10.5.5 Centrifuge Equipment

Hazards associated with centrifugation include mechanical failure and the creation of aerosols. To minimize the risk of mechanical failure, centrifuges must be maintained and used according to the manufacturer's instructions. Users shall be properly trained and operating instructions, including safety precautions, should be prominently posted on the unit.

Aerosols are created by filling centrifuge tubes, removing supernatant, and resuspending sedimented pellets. The greatest aerosol hazard is created if a tube breaks during centrifugation. Avoid using glass tubes wherever possible. To minimize the generation of aerosols when centrifuging BMs, follow these procedures:

- Use sealed tubes and safety buckets that seal with O-rings. Before use, inspect tubes, O-rings, and buckets for cracks, chips, erosions, bits of broken glass, etc. Do not use aluminum foil to cap centrifuge tubes because it may detach or rupture during centrifugation.
- Fill and open centrifuge tubes in a BSC. Avoid overfilling centrifuge tubes so that closures do not become wet. After tubes are filled and sealed, wipe them down with disinfectant.
- Make sure the sample tubes fit the adaptor pad in the buckets.
- Always balance buckets, tubes, and rotors properly before centrifugation.
- Avoid decanting or pouring off supernatant. A vacuum system with the appropriate in-line reservoirs and filters is recommended (see paragraph 10.5.7).
- Work in a BSC when resuspending sedimented material. Use a swirling rotary motion rather than shaking. If shaking is necessary, wait a few minutes for the aerosol to settle before opening the tube.
- Small low-speed centrifuges may be placed in a BSC during use to reduce the aerosol escape. High-speed centrifuges that potentially disrupt the air flow are not allowed to be operated in the BSC. Manufacturer's recommendations must be followed to avoid metal fatigue, distortion, and corrosion.
- Avoid the use of celluloid (cellulose nitrate) tubes with BMs. Celluloid centrifuge tubes are highly flammable and prone to shrink with age. They distort when boiled and can be highly explosive in an autoclave. If celluloid tubes must be used, appropriate chemical disinfectants are necessary for decontamination.

10.5.6 Blenders, Ultrasonic Disrupters, Homogenizers, Grinders, and Lyophilizers

Using any of these devices produces considerable aerosols. Blending, cell-disrupting, and grinding equipment shall be used in a Class II BSC when working with BSL-2 BMs and shall be decontaminated after use.

- Safety blenders are recommended for processing BMs. A towel moistened with disinfectant should be placed over the top of the blender during use. Before opening the blender jar, allow the unit to rest for at least one minute to allow the aerosol to settle. The device should be decontaminated promptly after use.
- Depending on the design of the lyophilizer, aerosol production may occur when material is loaded or removed from the lyophilizer unit. If possible, sample material

should be loaded in a BSC. The vacuum-pump exhaust should be filtered to remove any hazardous agents. After lyophilization is completed, the surfaces of the unit that have been exposed to the agent should be disinfected. If the lyophilizer is equipped with a removable chamber, it should be closed off and moved to a BSC for unloading and decontamination.

- Open ampoules containing liquid or lyophilized infectious culture material in a BSC to control the aerosol produced. Gloves shall be worn. To open, nick the neck of the ampoule with a file, wrap it in disinfectant-soaked towel, hold the ampoule upright, and snap it open at the nick.
- Reconstitute the contents of the ampoule by slowly adding liquid to avoid aerosolization of the dried material. Mix the container. Discard the towel and ampoule top and bottom as biohazardous waste. Ampoules should not be used for storing BMs in liquid nitrogen. The use of polypropylene tubes designed for cryogenic storage may eliminate this hazard. The researcher shall comply the procedure listed in KSC-ESC-3000, Chapter 10, Cryogenic Safety.

10.5.7 Vacuum Lines

Vacuum lines shall be protected with liquid disinfectant traps, HEPA filters, or filters of equivalent efficiency. Filters should be routinely checked and maintained or replaced as necessary. Filters are required to be disposed as biohazardous material.

10.5.8 Housekeeping

Routine housekeeping shall be maintained to minimize potential sources of contamination. Laboratory personnel are responsible for maintaining the cleanliness of laboratory benches, equipment, and areas that require specialized technical knowledge.

Additional laboratory housekeeping requirements include:

- Keep the laboratory neat and free of clutter. Surfaces should be clean and free of infrequently used chemicals, glassware and equipment.
- Do not block access to sinks, eyewash stations, emergency showers, exits, and fire extinguishers.
- Keep the workplace free of physical hazards. Aisles and corridors should be free of tripping hazards. Pay attention to electrical safety, especially as it relates to using extension cords, properly grounding equipment, and avoiding electrical hazards in wet areas.
- Clean and disinfect laboratory equipment before it is released for repair or maintenance.
- Properly dispose of waste chemicals, biological waste, and nonhazardous waste.

10.5.9 Packaging and Transportation of BMs On- and Off-Site

To ensure the safety of employees, the public, and the environment, package and transport all biological materials in a way that maintains the integrity of the material and ensures its containment during normal transport conditions.

10.5.9.1 Transportation within the same building or between different buildings:

Follow these guidelines when transporting BMs:

- Package samples in a sealed leak-proof primary container (i.e., plastic screw-top conical tube), which is securely positioned in a secondary leak-proof and closable container (i.e., cooler or ice chest). The outside of the secondary container shall have a clearly visible biohazard symbol.
- Attach a plastic pouch containing a list of contents and emergency information (i.e., PI phone number) to the cooler.

10.5.9.2 Transportation and shipment off-site

Transportation and shipping BMs over public roadways is regulated by national and international transportation rules. This includes specific procedures for the correct packaging of these materials (http://www.un3373.com/info/regulations/), and the necessary documentation, labeling, and permits, if applicable. For more information about specific shipment requirements, contact the BSO.

- Using private cars to transport BMs is prohibited.
- External transport security should ensure appropriate authorization and communication between facilities before, during, and after external transport which may involve a commercial transportation system. The United Nations Model Regulations for the Transport of Dangerous Goods, includes provisions addressing the security of dangerous goods, including infectious substances, during transport.
- Many countries request import and export permits for biological materials be filed before the transfer of such specimens is authorized. These procedures allow for registering and tracking of materials entering or leaving a country. The requirements and procedures to obtain such permits are found in the websites of CDC Import Permit Program (http://www.cdc.gov/od/eaipp/), and APHIS/USDA (https://www.aphis.usda.gov/aphis/ourfocus/importexport or https://www.aphis.usda.gov/aphis/resources/permits).

10.5.10 Personal Protective Equipment

Personal protective equipment (PPE) should be worn following the requirements in KNPR 8715.5 when handling BMs. Appropriate PPE is based upon the potential hazards and

risks associated with the BMs. Common PPE includes lab coats, gloves, ANSI-rated safety glasses, goggles, and face shields.

Using PPE is required in procedures where there is a likelihood of contamination with any BMs. Appropriate PPE in various sizes shall be available and easily accessed by laboratory personnel.

10.5.10.1 Gloves

- Employees must wear gloves when they may have hand contact with blood, other potentially infectious materials, when handling or touching contaminated items or surfaces, or when they have nonintact skin.
- Gloves should fit the user's hands comfortably.
- Nitrile gloves must be worn to handle BMs.
- When handling certain BMs identified in an HMI assessment, and when performing dissection or surgical procedures, two pairs of glove should be worn.
- Reusable heavy-duty gloves made of latex or nitrile may be used when cleaning contaminated items or surfaces with caustic disinfectants.
- Replace disposable (single-use) gloves, such as surgical or examination gloves, as soon as practical when contaminated, or as soon as feasible if they are torn, punctured, or when their ability to function as a barrier is compromised.
- Do not reuse disposable (single-use) gloves.

10.5.10.2 Gowns, aprons, and other protective body clothing

Wear appropriate protective clothing such as gowns, aprons, laboratory coats, clinic jackets, or similar outer garments when handling BMs or working on potentially contaminated items or surfaces.

- Employees who handle contaminated clothing shall wear protective gloves and other appropriate PPE.
- Contaminated clothing is disposed of as biohazardous waste (or medical waste if handling human samples) in accordance with KNPR 8500.1 and KSC-ESC-3000, Chapter 48, Waste Management – Biomedical Waste.
- If applicable, contaminated laundry may be handled by a qualified laundry service. Contact the BSO for information.
- Do not sort or rinse contaminated laundry in the location of use.
- Place and transport contaminated laundry in red biomedical waste bags or in containers labeled and color coded.

11. METHODS OF DECONTAMINATION

Decontamination is defined as the reduction of microorganisms to an acceptable level. Disinfection or sterilization are the most common methods applied to reach this goal. Disinfection is used when the acceptable level of microorganisms is defined as being below the level necessary to cause disease. Therefore, viable microorganisms may still present. In contrast, sterilization is defined as the complete killing of all organisms present. Depending on the circumstances and tasks, decontamination of a surface (e.g., a lab bench) is accomplished with a disinfectant, while decontamination of biomedical waste is done by sterilization in an autoclave.

In order to select the proper method and tools, it is important to consider the following aspects: type of biohazardous agents, concentration and potential for exposure; and physical and chemical hazards to products, materials, the environment, and personnel. Decontamination methods fall into four main categories: heat, liquid chemicals, vapors and gases, and radiation.

Sterilization can be achieved by using ethylene oxide (EtO), paraformaldyhate (PFA), and wet heat (steam sterilization in an autoclave). The use of EtO and PFA shall comply with OSHA Standard 1910.1047 and 1910.1048, respectively. EtO sterilization is mainly used to sterilize medical and pharmaceutical products that are not suitable for conventional high-temperature steam sterilization. Formaldehyde gas is mainly used for space decontamination, such as inside the BSC. Some liquid chemicals can be applied for surface sterilization, if used in the right concentration and contact time.

11.1 Surface Decontamination

- Bench-top and BSC surfaces shall be chemically disinfected in a solution of 70% ethanol, or bleach (NaCIO) (10% volume/volume% [v/v] diluted bleach) followed by sterile water (or 70% ethanol), for at least 15 minutes before and after handling BMs.
- Wipe door handles and water faucets with a 70% ethanol solution at the end of each work day or whenever a potential contamination is suspected.
- Glassware and other reusable items shall be chemically decontaminated (with 10% bleach solution, if compatible with the material, or 70% ethanol) and allowed to stand for 15 minutes, followed by rinsing with copious amounts of cold water and autoclaved prior to being returned to service.

11.2 Decontaminating Liquid

- Decontaminating liquids shall be made by adding undiluted bleach (NaCIO, 6% weight/volume% [w/v] stock concentration) to a final concentration of at least 0.3% w/v NaCIO or 10% dilution. Mix well and allow the solution to stand for 15 minutes before disposing of in accordance with an approved Process Waste Questionnaire Technical Response Package (PWQTRP).

- Do not autoclave large quantities of bleach solution. If it is impractical to rinse items, the NaCIO (bleach) should be neutralized by adding 1 milliliter of 5% sodium thiosulfate per milliliter of 5% hypochlorite ion.
- Small quantities of liquid or liquid waste that are not compatible with NaCIO should be autoclaved for at least 30 minutes, using slow exhaust, before being disposed of in the drain (with an approved PWQTRP).

11.3 Steam Sterilization/Autoclaving

Decontamination of BMs or associated supplies using autoclaves shall be operated and maintained in accordance with the manufacturer's manual. An operation procedure is listed in the Appendix G.

- Autoclave cultures and stocks of infectious agents, consumables used in the manipulation of said cultures, recombinant DNA (rDNA), human, and infectious animal tissue waste, and reusable lab ware.
- DO NOT AUTOCLAVE items contaminated with solvents, volatile or corrosive chemicals, or items containing carcinogens, mutagens, or teratogens.
- Various devices can be used to indicate the proper functioning of an autoclave, ranging from autoclave tape to chemical and biological indicators. DO NOT use autoclave tape (the least reliant) as the only indicator of sterilization and decontamination.
- Personnel must receive training before using the autoclave.

11.4 Formaldehyde Gas for Space Decontamination

Formaldehyde gas at a concentration of 0.3 grams/cubic foot for four hours is often used for space decontamination, such as BSC decontamination. Gaseous formaldehyde can be generated by heating flake paraformaldehyde (0.3 grams per cubic foot) in a frying pan, thereby converting it to formaldehyde gas. The humidity must be controlled and the system works optimally at 80% relative humidity. This method is effective in killing microorganisms, but toxicity issues are present. Formaldehyde gas decontamination shall only be performed by a certified contractor.

12. WASTE MANAGEMENT

A Process Waste Questionnaire (PWQ), Form KSC26-551, must be completed and submitted to the ESC Safety, Health, and Environmental (SHE) engineer before generating biohazardous waste. After reviewing the PWQ, a PWQTRP shall be used to document the required waste handling and disposal methods. Disposals of biohazardous wastes must comply with the requirements in KSC-ESC-3000, Chapter 48, Waste Management – Biomedical Waste, as well as the requirements defined in the PWQTRP for the specific waste.

Biological wastes shall be deposited in an approved biohazardous (red) bag for offsite decontamination or disposal in a permitted facility by steam sterilization or incineration. Materials to be stored or decontaminated onsite in an area outside of the immediate laboratory are to be placed in a durable leak-proof container and closed for transport from the laboratory. Materials to be decontaminated offsite shall be managed in accordance with KSC-ESC-3000, Chapter 48, Waste Management – Biomedical Waste, and applicable Federal and State regulations before being removed from the site.

BSL-2 BM waste shall be disposed in a covered biohazardous waste container. Disposables such as pipette tips, test tubes, Petri plates, gloves, sleeves, gowns, and shoe covers are to be placed in a biohazardous waste container with a cover; and when the biohazardous waste container is filled, sealed and transported to a Biohazardous Waste Storage Area (BWSA) before it is shipped offsite for disposal (see KSC-ESC-3000, Chapter 48, Waste Management – Biomedical Waste).

Volatile or organic solvents (such as fixatives) are toxic to biological materials and do not need to be chemically decontaminated or autoclaved. They are to be disposed of in accordance with an approved PWQTRP.

13. BIOSECURITY AND BIOETHICS

The purpose of biosecurity measures is to reduce the risk of unauthorized access, loss, theft, misuse, diversion, or intentional release of BMs; and to provide a framework for continuous awareness-raising for biosafety, laboratory biosecurity, ethical code of conduct, and training within the facility.

13.1 BM Information Management

The use and storage of BMs should be limited to clearly documented areas in ALCs/HMIs as well as BM Inventory List maintained in the VERTÉRE Database. The list includes the information of organism type, host, name, strain, BSL level, location, origin, and associated HMI. Internal material transfer requires updated documentation, including ALCs and HMIs, if new lab capabilities or mitigations are required, as well as an updated Inventory List. Confidential information on human specimens is required to be maintained in double-locked restricted areas. However, accountability does not necessarily imply the identification of exact quantities of BMs.

13.2 Natural Disasters

Natural disasters threatening the containment and biosecurity of laboratories in regions at geological risk (earthquakes, hurricanes, floods, tsunamis, etc.), pose risks of potential exposure of employees, the general population, and the environment to BMs. Mitigation of possible negative outcomes caused by the release of BMs during natural adverse events shall be addressed in the LMP.

13.3 Biosecurity

The LMs, PIs, lab personnel, and the BSO or designated SHRB members should communicate, collaborate, and maintain the correct ethical balance for the activities performed. Comprehensive bioethical reviews by the BSO should be carried out at a Biological Hazard Assessment (BHA) review and documented before final decisions are reached on the authorization of the study, or before publication of data.

13.4 Bioethics

The fundamental principles described in the Belmont Report, namely autonomy, beneficence, and justice, shall be followed when conducting biomedical and behavioral research involving human subjects. The Belmont Report established by the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research can be found at the website of U.S. Department of Health & Human Services (http://www.hhs.gov/ohrp/regulations-and-policy/belmont-report/index.html). The scope of bioethics may expand to modern biotechnology techniques including cloning, gene therapy, genomics research, the translation of research findings into health care, and manipulation of basic biology through altered DNA, XNA, and proteins. The Office of Clinical Research and Bioethics Policy may be consulted for potential bioethics issues identified during HMI assessment.

APPENDIX A. REQUIRED INFORMATION IN HMI FORM FOR BHA

The LMs and PIs should provide the following information on BMs in HMI form:

Primary Laboratory/Location:

Other Laboratories involved:

Name(s) of Personnel Handling Proposed Materials:

Types of Biological Materials (strain name and catalog #):

Origin of biological materials (Previous owner or Vendor):

Storage Method and Location:Will any DNA molecules or genetic modified organisms be used in the project?

If YES, please fill the following information:

Host (e.g., HEK293):

Vector (e.g. Lentivirus):

Source of Gene (e.g., jellyfish):

Type of Gene (e.g., marker):

Gene or Oligonucleotides Expressed (e.g., yes, Green Florescent Protein (gfp)):

If Gene is expressed, protein produced (e.g., Green Florescent Protein):

Brief experimental procedure (Please describe briefly the experimental procedure that will be used to manipulate the biological material, e.g. treatment, irradiation, transgenic technique, etc.):

APPENDIX B. BIOLOGICAL SAFETY INSPECTION CHECKLIST

1	A Spill kit shall be readily available.
2	An eyewash station shall be readily available.
3	Vacuum lines shall be protected with liquid disinfectant traps.
4	BSCs shall be recertified within expiration date.
5	No clustered objects are stored in BSCs.
6	Used disposable needles and syringes shall be carefully placed in conveniently located puncture-resistant containers used for sharps disposal in accordance with KSC-ESC-3000, Chapter 48, Waste Management – Biomedical Waste.
7	Biological waste materials shall be stored in a biohazard bag in a container with lid, and shall be packed for being removed from the facility for decontamination in accordance with KSC-ESC-3000, Chapter 48, Waste Management – Biomedical Waste.
8	A biohazard symbol shall be posted at the entrance to the laboratory when biological materials are present. Posted information must include: the laboratory's biosafety level, the responsible personnel, telephone number, and required procedures for entering and exiting the laboratory.

APPENDIX C. BIOLOGICAL SPILL CLEAN UP PROCEDURE

C.1 General Procedures

- Alert people the in immediate vicinity to leave the area and restrict access to the spill area.
- Notify supervisors and post a warning.
- Laboratories should allow aerosols to settle for 30 minutes before reentering.
- Put on PPE required for clean-up procedures and the safe use of disinfectant.
- Cover an area twice the size of the spill with disinfectant-soaked-paper towels, or surround the spill with dry disinfectant in accordance with label directions.
- Pour additional disinfectant solution onto the spill, starting at the perimeter and working inward from the edges of the towels. Avoid splashing.
- Allow at least 20 minutes contact time.
- Use forceps, tongs, or a broom to remove broken glass and other sharps; place the sharps in a biohazard sharps container.
- Remove towels and reclean area with disinfectant solution.
- Wipe down any contaminated stationary equipment or furniture twice with disinfectant-soaked paper towels.
- Decontaminate (using an autoclave or chemical disinfectant) reusable clean-up items and other equipment as appropriate.
- Dispose of the waste as biohazardous waste.
- Inform laboratory personnel when the clean-up is complete.

C.2 Spill Inside a Centrifuge

- Have a complete biological spill kit ready to go before starting the clean-up.
- Wear a lab coat, safety goggles, appropriate respiratory protection, and gloves during clean up.
- If the centrifuge is inside a BSC, allow the cabinet to run during clean up.
- Soak up spilled material with disposable paper towels (work surface and drain basin) and apply disinfectant with a minimum of 10 minutes contact time.
- Wipe up spillage and disinfectant with disposable paper towels.
- Wipe the walls, work surface and any equipment in the cabinet with a disinfectant-soaked paper towel.

- Discard contaminated disposable materials in biohazard bags and autoclave before discarding as waste.
- Place contaminated reusable items in biohazard bags, or heat resistant pans or containers with lids before autoclaving and further clean up.
- Expose materials that cannot be put in an autoclave to disinfectant, 20 minutes contact time, before removal from the BSC.
- Remove potentially contaminated protective clothing used during cleanup and place in a biohazard bag for further processing by disposal or laundry described in paragraph 10.6.9.
- Run the cabinet for at least 10 minutes after clean up and before resuming work.
- Inform users of the BSC and the laboratory manager and supervisor about the spill and successful clean up as soon as possible.

C.3 Spill outside the Laboratory (example – during transport)

- When a spill of BMs occur outside the laboratory in a public area, follow the instructions in Section 8. Do not attempt to clean up the spill without the proper PPE and spill clean-up materials.
- Always transport biohazardous materials in accordance with paragraph 10.6.9.

APPENDIX D. RISK GROUP CLASSIFICATIONS AND BIOSAFETY LEVEL ASSIGNMENTS

There are several systems for classifying human and animal pathogens according to the hazard they present to an individual and the community. Although these classifications differ, they are all based on the notion that some microorganisms are more hazardous than others. In general, the pathogenicity of the organism, mode of transmission, host range, availability of effective preventive measures or effective treatment are some of the criteria taken into consideration when classifying infectious agents. In the U.S.A., the most current classification is found in the NIH Guidelines for Research Involving Recombinant DNA Molecules. The human etiologic agents addressed in these guidelines are classified into four risk groups, with Risk Group 1 (RG-1) of low or no hazard and Risk Group 4 (RG-4) representing highly infectious agents. Determining the RG of a biological agent is part of the biosafety risk assessment and helps in assigning the correct biosafety level for work practices and containment. In general, RG-2 agents are handled at BSL-2, and RG-3 agents at BSL-3. However, certain RG-2 agents, depending upon the operation, may require BSL-3 conditions, and some RG-3 agents may be safely manipulated at a BSL-2. HIV is an example of a RG-2 agent, that depending on the task being performed, will be handled at BSL-2 or BSL-3. For more information, refer to Section 5 of this document or contact the biological safety officer.

Risk Group Levels are classified as:

Risk Group 1 (RG-1): A biological agent that is unlikely to cause disease in healthy workers or animals.
Risk Group 2 (RG-2): A pathogen that can cause human or animal disease but, under normal circumstances, is unlikely to be a serious hazard to laboratory workers, the community, livestock, or the environment, and for which preventive or therapeutic interventions are often available.
Risk Group 3 (RG-3): Agents that are associated with serious or lethal human or animal disease for which preventive or therapeutic interventions may be available (high individual risk but low community risk).
Risk Group 4 (RG-4): Agents that are likely to cause serious or lethal human or animal disease for which preventive or therapeutic interventions are not usually available (high individual risk and high community risk).

Correspondingly, the Biosafety Levels are classified as:

Biosafety Level 1 (BSL-1): A level that is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans and of minimal potential hazard to laboratory personnel and the environment. At this level, precautions against the biohazardous materials in question are minimal, most likely involving gloves and some sort of facial protection with no special primary or secondary barrier recommended, other than a sink for hand washing. It relies on standard microbiological practices with basic PPEs described in paragraph 10.5 of

this document. BSL-1 practices, safety equipment, and facilities are appropriate for work with defined and characterized strains of viable microorganisms not known to cause disease in healthy adult humans. *Bacillus subtilis* is a representative of these microorganisms. Many agents not pathogenic in healthy humans are, however, opportunistic pathogens that may cause disease in the young, the aged, and immunodeficient or immunosuppressed individuals. Animal pathogens can also infect other susceptible hosts, including humans.

Biosafety Level 2 (BSL-2): Practices, safety equipment, and facilities are applicable for work associated with the broad spectrum of indigenous moderate-risk agents present in the community and associated with human or animal disease of varying severity, posing moderate potential hazard to personnel and the environment. Hepatitis B virus, the Salmonella, and Toxoplasma species (spp.) are representative of microorganisms assigned to this containment level. BSL-2 containment is also appropriate when work is done with any human-derived blood, body fluids, or tissues where the presence of an infectious agent may be unknown. This also applies to animal tissues or blood when the presence of an infectious agent is unknown.

General Facility and Containment Guideline

The general containment guideline is summarized in Table 1. Personnel working with human-derived materials should refer to the organization’s Bloodborne Pathogens Exposure Control Plan for specific required precautions.

Table 1. General Containment Guidelines for BSL-1 and BSL-2

BSL	Agents	Practices	Primary Barriers and Safety Equipment	Facilities (Secondary Barriers)
1	Not known to consistently cause diseases in healthy adults.	Standard microbiological practices	None required	Laboratory bench and sink required.
2	Agents associated with human disease. Routes of transmission include percutaneous injury, ingestion, or mucous membrane exposure.	BSL-1 practice plus limited access and biohazard warning signs must be posted, "sharps" precautions must be enforced	Class I or II BSCs or other physical containment devices must be used for all manipulations of agents that cause splashes or aerosols of infectious materials. PPEs including laboratory coats, gloves, and face protection must be used as needed.	BSL-1 plus an autoclave must be available

APPENDIX E. RISK GROUP CLASSIFICATIONS AND PLANT BIOSAFETY-LEVEL ASSIGNMENTS

Only plant and plant cells with BSL-2P classification and lower shall be allowed in KSC laboratories or facilities.

Biosafety Level 1 – Plants and Plant Studies (BSL-1P): A level that is suitable for work involving transgenic plants where there is no evidence that the modified organism would be able to survive and spread in the surrounding environment and, if released, would not present an environmental risk. An example would be an experiment with transgenic potato plants containing cloned genes for insect resistance obtained from primitive potato cultivars. This biosafety level also applies to a plant study involving DNA-modified microorganisms that are not known to have any negative effects on either natural or managed ecosystems, such as using a transgenic *Rhizobium* strain containing *Agrobacterium* genes known to affect root colonization. The research activities typically are carried out using BSL-1 containment facilities, equipment, and practices.

Biosafety Level 2 – Plants (BSL-2P): A level that is suitable for work involving transgenic plants and associated organisms, which, if released, could survive and spread in the surrounding environment but would have a negligible effect or can be easily controlled. BSL-2P also applies to transgenic plant-associated microorganisms that are potentially harmful to the environment but manageable. BSL-2P whole-plant experiments involve (1) exotic infectious agents with recognized potential for serious, detrimental impact on ecosystems if handled without proper containments; (2) sequence-encoding potent vertebrate toxins introduced into plants or associated organisms; (3) the usage of the entire genome of an indigenous infectious agent or pathogen that may pose a health risk to lab personnel; (4) plants together with microorganisms or insects containing rDNAs; or (5) transgenic plants that may exhibit noxious weed or parasitic plant characteristics or may be capable of interbreeding with weeds, parasitic plants, or related species growing in the vicinity (Florida State-listed Noxious Weeds: http://plants.usda.gov/java/noxious?rptType=State&statefips=12).

BSL-1P/2P Facility Management and Containment Guideline

The general facility biosafety management guideline for plant studies is summarized in Table 2 and adheres to the NIH and USDA guidelines. The containment requirements and exposure control measures are consistent, whenever applicable, to those applied for BSL-1/BSL-2 agents, which are listed in this document and its appendixes.

Table 2. Facility Biosafety Management Guidelines for Plant Studies

BIOSAFETY LEVEL 1-P	BIOSAFETY LEVEL 2-P
Discretionary access	Access limited to individuals directly involved with experiments.
Procedures followed are appropriate for organisms.	A Greenhouse/environmental chamber manual must be available to advise of consequences and provide contingency plans
Record kept of experiments in facility	Records kept of experiments and movement in/out of facility.
	Containment required for movement in/out of greenhouse/environmental chamber
Biologically inactivate experimental organisms at the end of the experiment.	Biologically inactivate experimental organisms at end of experiment. Decontaminate gravel/environmental chamber periodically.
Pest control program	Pest control program
Provide appropriate caging and precautions for escape of motile organisms.	Provide appropriate caging and precautions for escape of motile organisms, if applicable
	Post sign for Biosafety Level 2 restricted experiment in progress with plant names, responsible person, and special requirements.

APPENDIX F. BIOSAFETY CABINETS

BSCs are not chemical fume hoods. They do not remove or control toxic, flammable, corrosive, or radioactive gases or vapors. A brief description of the different types of biosafety cabinets is as follows:

CLASS I BSC: The Class I BSC provides personnel and environmental protection, but no product protection. It is similar in air movement to a chemical fume hood, but has a HEPA filter in the exhaust system to protect the environment. In the Class I BSC, unfiltered room air is drawn across the work surface and personnel protection is provided by this inward airflow. In most cases, Class I BSCs are used specifically to enclose equipment (e.g., centrifuges, harvesting equipment, or small fermenters), or procedures (e.g. cage dumping, aerating cultures, or homogenizing tissues) with a potential to generate aerosols.
http://www.cdc.gov/od/ohs/biosfty/primary_containment_for_biohazards.pdf

CLASS II BSC: The Class II BSC provides personnel, environmental and product protection. Airflow is drawn around the operator into the front grille of the cabinet, which provides personnel protection. In addition, the downward laminar flow of HEPA-filtered air provides product protection by minimizing the chance of cross-contamination along the work surface of the cabinet. The Class II cabinet has four designs that differ in the amount of air that is recirculated or exhausted, and whether or not the BSC is hard-ducted to the ventilation system.

Two varieties of Class II BSCs are used on KSC. Both are adequate for manipulations of RG-2 pathogens. CLASS II TYPE A/B3 cabinets recirculate 70% of the internal air and exhaust 30% of filtered air into the laboratory. CLASS II TYPE B (A2) cabinets either recirculate 30% of internal air and exhaust 70% of filtered air through a duct to the outside atmosphere, or exhausts 100% of the air. Because of the greater safety margin, small amounts of nonvolatile chemical carcinogens, antineoplastic drugs, or radioactive materials may be used in this cabinet. The BSO should be consulted to aid in the selection of correct BSC for specific tasks.

The ultraviolet lamps within some BSCs provide only limited ability to inactivate microbes. Efficacy is limited to exposed surfaces and penetration of organic material is poor. Effectiveness also decreases as the lamp ages. Furthermore, exposure to the ultraviolet light may cause eye damage. Ultraviolet lamps are not recommended to be the sole source of decontamination of BSC surfaces.

- To ensure sterility inside the BSC and to establish proper air flow for containment, the blower should be turned on at least 10 minutes before infectious or potentially infectious materials are placed in the BSC. The BSC airflow (Magnehelic) gauge should be checked (reading is equal to approximately 0.5 inches) to ensure proper operation of the cabinet before placing any materials into it. Readings indicate relative pressure-drop across the HEPA filter. Higher readings may, therefore, indicate filter clogging. Zero readings may indicate loss of filter integrity. If a problem is indicated, contact the LMs.

- BSC work surfaces shall be cleaned with an approved disinfectant before each use and as required by the research requirements.
- Do not place items on the front or rear grille of a BSC. Disrupting the airflow into the front grill allows contaminated air from inside the cabinet to flow into the lab or directly at the person using the cabinet. It also allows nonsterile air from the room to enter the cabinet. Any disruption of the airflow in the cabinet decreases its effectiveness.
- Before manipulating BMs or potentially BMs, required equipment and materials should be placed in the BSC. Fewer entries into the BSC results in less disruption of the airflow and a more-effective level of containment. Work should be performed on the center of the work surface whenever possible, with work progressing outward from clean to dirty (contaminated).
- After manipulating infectious or potentially infectious agents or animals, containers and cage filter-tops are to be closed before being removed from the BSC.
- The surface of equipment used in manipulations (pipettors, balances) shall be wiped down with disinfectant before being removed from the BSC.
- Biohazardous waste and disposable items shall be left in the cabinet until properly decontaminated or placed in the appropriate biohazardous waste container for offsite disposal.
- After the cabinet has been emptied, wipe exposed surfaces (including the front grille and splash area) with disinfectant. Allow the blower to run for at least 10 minutes to purge any aerosols from inside the cabinet before shutting off the blower.
- No modifications shall be made to the BSC.

Operation Procedure

a. Startup

(1) Turn on the blower and the fluorescent light.

(2) Wait at least ten minutes before loading equipment. This is to purge the BSC of contaminated air.

(3) Check grills for obstructions.

(4) Adjust the sash according to the KEMCON certification sticker to proper position.

(5) RESTRICT traffic in the BSC vicinity.

b. Loading Materials and Equipment

(1) Load only items needed for the procedure.

(2) Ensure that the rear or front exhaust grills are free from obstruction.

(3) Disinfect the exterior of containers prior to placing them in the BSC.

(4) Arrange materials to minimize movement within the cabinet.

(5) Arrange materials within the cabinet from CLEAN to DIRTY (or STERILE to CONTAMINATED).

(6) Materials should be placed at least six inches from the front BSC grill.

(7) Never place nonsterile items upstream of sterile items.

(8) Maintain the BSC sash at proper operating height according to the sticker.

c. Work Technique

(1) Wash hands thoroughly with soap and water before and after any procedure.

(2) Wear gloves and a laboratory coat; use aseptic technique.

(3) Avoid blocking the front grill. Work only on a solid, flat surface; ensure that any chair in use is adjusted so armpits are at the elevation of the lower window edge.

(4) Avoid rapid movement during procedures, particularly within the BSC, but also in the vicinity of the BSC.

(5) Move hands and arms straight into and out of the work area.

d. Final Purging and Wipe-down

(1) After completing work, run the BSC blower for ten minutes before unloading materials from the cabinet. Disinfect the exterior of containers BEFORE removal from the BSC.

(2) Decontaminate interior work surfaces of the BSC with an appropriate disinfectant.

APPENDIX G. AUTOCLAVE PROCEDURE

Operation Procedures

The following are guidelines on proper use of an autoclave and do not replace hands-on training.

a. BEFORE Autoclaving
   (1) Review the operator's manual for instructions as different makes and models of autoclaves have different controls.
   (2) Wear appropriate PPE while loading and unloading the autoclave, including heat-resistant gloves, lab coat, and eye protection. A face shield should be worn if a splash hazard is present.
   (3) Use autoclavable polypropylene/polyethylene biohazard bags ONLY.
   (4) Use a heat-resistant secondary container to retain any leakage that may occur.
   (5) DO NOT overfill bags or the autoclave chamber as this decreases its effectiveness.
   (6) Leave bags unsealed to allow steam penetration.
   (7) Fill liquid containers only half full, and loosen caps or use vented closures.
   (8) DO NOT autoclave liquid and dry material together.

b. DURING Autoclaving
   (1) Use appropriate cycle times for the items being autoclaved:
      (a) Sterilizing Clean Materials: 30 min. at 121°C and 15 psi
      (b) Decontaminating Waste: 60 min. at 121°C and 15 psi
      (c) Dense Loads: lengthen running time
      (d) Liquids: use slow exhaust
      (e) Glassware: use fast exhaust
   (2) Segregate autoclave loads (infectious waste, liquid, or labware).
   (3) DO NOT leave autoclaved material in the autoclave overnight.

c. AFTER Autoclaving
   (1) Make sure the pressure has gone to ZERO before opening the door.
   (2) Allow materials to cool down for 15-20 minutes prior to their removal.
   (3) Use extreme caution when opening an autoclave door – there still may be steam inside the chamber after the pressure has dropped to zero, which can cause severe burns.